A Synopsis submitted to

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Biosciences (Microbiology).

Subject	Biosciences (Microbiology)
Faculty	Science
Titles of the thesis	"PRODUCTION OF BACTERIAL LIPASE AND ITS ROLE IN BIOREMEDIATION OF OIL CONTAMINATED EFFLUENT"
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INTRODUCTION

Treatment of polluted wastewater with Fat and oil involves physical/chemical methods such as filtration, settling, precipitation, and other chemical treatment. These methods have limited application not only because of less efficiency but they introduce secondary pollutants during the 'remediation' process which are quite costly to run, maintain and clean up. Microbial degradation is an environment friendly and cost - competitive alternative to chemical decomposition processes. Moreover microbial degradation can also detoxify the effluent effectively without leaving any residues.

Biological treatment offer a simple and cost effective ways of bioremediating oily effluents, however it also carries many inherent difficulties, such as maintaining the microbial population necessary to degrade a certain compound, and the necessary growth conditions (i.e. proper temperature, oxygen availability, moisture levels, pollutant levels, and pH) for the required microbe. These factors can limit the overall success of bioremediation.

The application of microbial enzymes instead of whole microbe in bioremediation can limits the mentioned difficulties and enhancing the bioremediation efficiencies. Enzymes are able to act in a large range of environmental conditions and remain active even if these conditions quickly change. Enzymes often are able to work in multiple environments, especially if they are immobilized. This makes the enzymes even more resistant to harsh environments and enables the enzymes even to be recovered and recycled after they are no longer needed.

The aims of this study are to identify and investigate the role of lipase produced by microorganisms in bioremediating industrial effluents. The interest in lipase arises due to the ability of these enzymes to catalyze the hydrolysis as well as synthesis of fatty acid esters. Lipases act on a variety of substrates including natural oils, synthetic triglycerides and esters of fatty acids. Many lipases show varied substrate and positional specificities and find use in various industries like, food, chemical, pharmaceutical, cosmetic, leather and detergent.

OBJECTIVES

a) To isolate the lipase producing microorganisms, able to hydrolyze oil & fats.
b) To isolate, purify and characterize the selected enzyme,
c) To optimize enzyme activity,
d) To optimize enzyme production and,
e) To carry out field trials on effluents.

RESEARCH SUMMARY

SAMPLE COLLECTION AND ISOLATION

Samples of oil contaminated soil were collected from different area surrounding Surat, Gujarat, India. For isolation of lipase producing organisms, tributyrine agar plats were used. Total of about 15 bacteria designated as BN 1-15 and 3 fungi designated as BNS
1-3 were isolated from soil samples of three different areas.

SCREENING OF THE ISOLATES FOR LIPASE PRODUCTION

Total of 15 bacteria and 3 fungi obtained were screened for their ability to produce lipase. Eight bacterial and one fungal isolates showing good extracellular lipase activity were isolated on tributyrine agar plates from oil contaminated sites. Out of which BN-3 bacterial spp. showed maximum lipase activity in the production medium (Basal medium) using olive oil as substrate.

IDENTIFICATION OF THE ISOLATES

Bacterial isolates BN-1, BN-2 & BN-3 were identified as *Pseudomonas aeruginosa Pseudomonas* spp. and *Pseudomonas stutzeri,* respectively by biochemical as well as 16s rDNA sequencing.

OPTIMIZATION OF PHYSICO-CHEMICAL PARAMETERS FOR MAXIMUM LIPASE PRODUCTION

Influence of Various Environmental Conditions on Growth and Lipase Production

The effect of Inoculum sizes (10^5 -10^9 cells/ml), substrate concentration- (1, 2, 4 ,5 and

10 gm%),temperatures (27°C - 45°C), pH (5 - 9) and mode of cultivation (static and shaking) on growth and lipase production were examined, from 24 to 96 h.
Maximum lipase activitywas found between 48 hrs to 72 hrs at 35°C temperature, at pH8 and, at 2gm% substrate concentration on a rotary shaker with 10^6 cells/mlinoculum size of *Pseudomon asaeruginosa*.

Effect of oil and carbon sources on lipase Production

Effect of four different oils as carbon sources were tested. Coconut oil,ground nut oil, sunflower oil and cotton seed oil at a final concentration of 1% (w/v) were tested, While the other parameters were kept constat.

Effect of nitrogen sources on lipase Production

Effect of four different nitrogen sources viz. urea, yeast extract, tryptone and $NaNo_3$ were Tested at a final concentration of 0.2 % (w/v). The other parameters were constant.

Effect of various detergents on lipase activity

Three different detergents like DMSO, SDS and Tween- 80, at a concentration of 1% were incubated at 35^0C for 1 hour with enzyme and then enzyme activity was then estimated.

Effect of various metal ions on lipase activity

Six metal ions like Ca^{+2},Na^{+2},Mg^{+2},K^{+},Fe^{+2}andZn^{+2} were tested. The enzyme was incubated for an hour at 35^0C in presence of 1mM of various ions salts. Then enzyme activity was then estimated.

LIPASE PRODUCTION WITH OPTIMISED MEDIUM AND PARAMETERS, SEPARATION AND MOLECULAR WEIGHT DETERMINATION.

Lipase was produced with optimized medium and process parameters in laboratory, which reveals that the isolates produced lipase in significant amount. Which was separated by column chromatography and molecular weight was determined by SDS PAGE.

conclusion

The results obtained showed successful isolation and identification of three different bacterial Species like, *Pseudomonas aeruginosa, Pseudomonas* spp. and *Pseudomonas stutzeri*. *Pseudomonas aeruginosa* showed maximum lipase activity and was used for lipase production. Which can be further used for bioremediation of oil contaminated effluents.

Work in progress:

Application of the organism for bioremediation of oil contaminated effluents.

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